Opioid Peptides: Potential for Drug Development

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Abstract

Opioid receptors are important targets for the treatment of pain and potentially for other disease states (e.g. mood disorders and drug abuse) as well. Significant recent advances have been made in identifying opioid peptide analogs that exhibit promising *in vivo* activity for treatment of these maladies. This review focuses on the development and evaluation of opioid peptide analogs demonstrating activity after systemic administration, and recent clinical evaluations of opioid peptides for possible therapeutic use.

Introduction

The endogenous ligands for opioid receptors are peptides. Since their initial discovery in the 1970s, there have been extensive studies of the structure-activity relationships (SAR) of opioid peptides for each of the opioid receptors (see refs. [1, 2] for reviews). However, the evaluation of opioid peptide analogs *in vivo* has been limited, typically to examination of activity following central (intrathecal, i.t., or intracerebroventricular, i.c.v.) administration. Relatively few opioid peptide analogs have been evaluated following more clinically relevant systemic (e.g., by subcutaneous, s.c., or oral, p.o.) routes of administration. This review focuses on reports of the analgesic and other behavioral effects of opioid peptides in animals following systemic administration, structural modifications that enhance the systemic activity of opioid peptide analogs, and also recent clinical trials of opioid peptides. The reader is referred to other recent reviews [2-5] for more detailed discussions on the development of these and other opioid peptides.

Development of systemically active opioid peptides

Given the importance of mu opioid receptor (MOR) agonists such as morphine as analgesics, the primary focus for opioid peptide analog development has been for the treatment of pain, although a few opioid peptides have been examined for other applications (see Table 1 and below). The emphasis in the majority of cases has been to induce analgesia through activation of opioid receptors in the central nervous system (CNS), although peripheral opioid receptor activation, particularly of kappa opioid receptors (KOR) [6], can also mediate analgesic activity (see below). In order to develop opioid peptide analogs as potential drugs they must be active after dosing by a clinically relevant route, typically by a form of systemic administration. There has been interest in examining opioid peptide

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analogs for systemic, including oral, activity in antinociceptive assays in animals for more than 20 years [7], with a number of peptides identified recently with improved pharmacokinetic properties. This review discusses these advancements in recent years in the search for opioid peptide analogs that are active after systemic administration.

Opioid peptides exhibiting activity after systemic administration in animals

An early successful demonstration of opioid peptides that exhibited activity after systemic administration were analogs of dermorphin (Figure 1), an endogenous opioid peptide obtained from amphibian skin that exhibits high selectivity for MOR. An early report [7] described analogs of the N-terminal tetrapeptide, which appears to be the minimum sequence from dermorphin required for agonist activity, that contained D-Arg at position 2 and that exhibited antinociceptive activity after both s.c. and oral administration. More recently, the dermorphin tetrapeptide analog ADAMB (Figure 1) was designed by incorporating modifications, including N-terminal quanidylation, from several dermorphin tetrapeptides that exhibited weak oral analgesic activity. ADAMB was 4- and 38-fold more potent as an analgesic than morphine following oral and s.c. administration, respectively (see Table 2), and lasted more than twice as long [8]. While the peptide was reasonably potent after oral administration (ED_{50} = 5.8 mg/kg in mice), its potency by this route was 65-fold lower than observed after s.c. administration, suggesting relatively low bioavailability by the oral route.

The best studied of the tetrapeptide analogs containing D-Arg^2 is Dmt-DALDA (Dmt-D-Arg-Phe-LysNH$_2$, Dmt = $2'$,$6'$-dimethyltyrosine; see ref. [4] for a review). This peptide is much more potent and demonstrates longer lasting analgesic activity than morphine when administered subcutaneously. Tolerance developed to this peptide's analgesic activity, but interestingly it exhibited minimal cross tolerance in morphine-tolerant mice. Dmt-DALDA is transported across Caco-2 monolayers, a model of the intestinal barrier, suggesting that this peptide could be orally absorbed. Since blockade of delta opioid receptors (DOR) can reduce morphine tolerance [4], Dmt-DALDA was linked to the DOR antagonist TICP[Ψ] (Tyr-TicΨ[CH$_2$HN]Cha-Phe, Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Cha = cyclohexylalanine) via an ethylenediamine linker at the C-termini in an effort to develop a MOR agonist/DOR antagonist producing minimal tolerance and which can cross the blood-brain barrier (BBB). The resulting peptide exhibited analgesic activity following s.c. administration comparable to morphine (Table 2), but with a longer duration of activity and substantially less tolerance [4].

Notably, recent reports suggest these opioid peptides containing D-Arg^2 have an unusual pharmacological profile that involves the release of endogenous opioid peptides, particularly the endogenous KOR ligands the dynorphins. This could possibly result in a more favorable side effect profile by minimizing liabilities such as respiratory depression and dependence associated with traditional opioid analgesics (see ref. [9] for a review).

Structural modifications similar to those used in ADAMB, including N-terminal guanidinylation, have been made to the MOR-selective endogenous mammalian opioid peptide endomorphin-1 to enhance BBB penetration (see Figure 1) [10]. The four analogs that contained D-Ala^2 along with guanidindo-Tyr-D-Pro-Gly-Trp-Phe(p-Cl)NH$_2$ each exhibited modest antinociception after systemic administration (see Table 2) which was blocked by naloxone administered i.c.v. but not by the peripherally-restricted antagonist naloxone methiodide, suggesting that these peptides produced their effects through MOR in the CNS.

The Dmt-Tic pharmacophore has been the basis of extensive SAR studies for opioid receptor interaction, particularly for DOR [11]. The DOR system is associated with the

regulation of emotional states (e.g., depression and anxiety), and although nonpeptide DOR agonists have shown antidepressant and anxiolytic activity, they also produce convulsions [12]. The peptidomimetic Dmt-Tic derivative UFP-512 (Figure 1) is a DOR selective agonist that has been examined *in vivo* [13-15]. UFP-512, but not the DOR agonist Dmt-Tic-NHCH$_2$-Bid (UFP-502, Bid = 1H-benzimidazole), exhibited antidepressant-like activity in the forced swimming test (Table 2) [14], suggesting that the CH$_2$CO$_2$H functionality conferred favorable pharmacokinetic properties, facilitating peptide transport across the BBB. Notably, tolerance did not develop to the antidepressant-like activity in this assay after 7 days of chronic treatment [13]. This compound also exhibited anxiolytic activity in two models following intraperitoneal (i.p.) administration [14]. Further studies of this peptidomimetic in a rat model of Parkinson's disease indicate a complex mechanism of action, with lower doses facilitating locomotor activity while higher doses worsened Parkinsonism-like symptoms, suggesting UFP-512 behaved as a partial agonist [15].

In contrast, the Dmt-Tic derivative Dmt-Tic-Lys-CH$_2$-Ph (MZ-2) is an antagonist at both MOR and DOR, blocking centrally administered morphine after both s.c. and oral administration [16]; after oral administration it was approximately 4-fold less potent than naloxone at antagonizing morphine in the hot plate test (Table 2). When MZ-2 was administered daily at a low dose (0.01 mg/kg s.c.) that did not prevent morphine- or DOR agonist-induced antinociception, it prevented the development of tolerance to morphine, consistent with its antagonist activity at DOR. Subsequently it was shown that oral administration of this peptidomimetic (10 mg/kg/day) could also reduce weight gain and improve other obesity-related factors in obese mice [17].

Recently there has been an interest in bi- and multifunctional opioid peptide ligands with activity at more than one target (see ref. [4] for a review). One approach has been to develop peptides possessing both opioid agonist and neurokinin 1 antagonist activities, since a neurokinin 1 antagonist was shown to attenuate the development of morphine tolerance (see ref. [4]). One of these peptides, TY005, has recently been evaluated *in vivo* for antinociceptive activity against nerve injury-induced hypersensitivity (i.e. antihyperalgesia) following i.v. administration [18]. It was found to be more effective than morphine in this model and did not lead to the development of analgesic tolerance.

Derivatives of the KOR endogenous opioid peptide dynorphin A have also been examined for analgesic activity following systemic administration. The dynorphin A fragment dynorphin A-(1-13) has been shown to exhibit antinociception in primates and humans in addition to in rodents (see ref. [3] for a review). The dynorphin A-(1-8) analog E2078 has been the most extensively studied in animals, and has also been administered to humans as an analgesic (see ref. [3] for a review). A dynorphin A-(1-7) analog stabilized to metabolism, SK-9709 (Figure 1), also exhibited antinociceptive activity following systemic (s.c.) administration (Table 2) [19]. In addition, YFa, designed as a chimeric peptide between Met[5]-enkephalin-Arg[6]-Phe[7] and FMRFa (Phe-Met-Arg-Phe amide) with structural similarities to dynorphin A, produced KOR-mediated antinociception in rats following i.p administration without producing tolerance [20], although large doses (40 mg/kg) were required. Structural modifications, i.e. D-Ala[2] and/or Phe(*p*-Cl)[4] incorporated into YFa to enhance metabolic stability and improve BBB penetration, resulted in enhanced analgesic potency following i.p. administration (see Table 2), but altered the opioid receptor selectivity [21, 22]. Like the parent peptide, these analogs did not produce antinociceptive tolerance. [NMeTyr[1]]dynorphin A-(1-13) and its C-terminal amide derivative have been shown to enhance morphine-induced antinociception in morphine-tolerant rats after systemic administration [23]. Interestingly, although the compounds were not active after s.c. administration, they were active following pulmonary delivery.

While the primary emphasis in the *in vivo* evaluation of opioid peptides has been for their antinociceptive activity, opioid peptide analogs have other potential therapeutic applications (see Box 1). We have recently demonstrated that the dynorphin A analog zyklophin (Figure 1), which is a highly KOR selective antagonist containing modifications that stabilize it to metabolism, is not only active after systemic (s.c.) administration but can also antagonize KOR in the CNS for a finite duration [24]. This peptide could have advantages over nonpeptide KOR-selective antagonists, which exhibit unusually prolonged antagonist activity (weeks after a single dose) [3]. Zyklophin also blocked drug seeking behavior in a model of stress-induced reinstatement of cocaine abuse, suggesting that such KOR peptide antagonists could have potential therapeutic applications in the treatment of drug abuse.

Box 1

Outstanding issues in the development of opioid peptides as therapeutic agents

1. Delivery of peptide therapeutics and increasing bioavailability

   The limited bioavailability of most peptides following oral administration has limited the development of opioid peptides as therapeutic agents. The demonstration of oral activity at reasonable doses for some opioid peptides suggests the possibility in selected cases to develop orally active opioid peptides, but it will be necessary to explore other routes of administration for a number of therapeutically relevant peptides. The demonstrated activity of dynorphin analogs following inhalation [23] suggest that it may be possible to administer these peptides by other methods not involving injection that could increase acceptance of such therapeutic agents.

2. Potential effects of species differences on blood-brain barrier permeability

   The recent results for the central vs. peripheral activity of MMP-2200 in mice vs. rhesus monkeys [27.28] illustrates one of the challenges in developing centrally acting peptides. Because of their polar nature, peptides often cross biological membranes by mechanisms other than passive diffusion, including via specific transporters (see ref. [25]). Thus species differences could have a greater impact on the distribution of peptides than for more hydrophobic small molecules that penetrate the CNS by passive diffusion. Understanding species differences in blood-brain barrier transport will also be important in the development of opioid peptide ligands restricted to the periphery.

3. Side effect profiles of opioid peptides compared to corresponding nonpeptide opioid ligands

   Side effects, e.g. the convulsant activity of small molecule DOR agonists, have limited the therapeutic development of some nonpeptide opioid ligands. In some cases distinct differences have been demonstrated for opioid peptide ligands compared to their nonpeptide counterparts, suggesting peptide opioid ligands could exhibit different side effect profiles from small molecule ligands. The high specificity of peptide ligands for opioid receptors can minimize off-target side effects compared to small molecule ligands, and therefore additional advantages of opioid peptide vs. nonpeptide ligands in terms of side effect profiles could emerge as more *in vivo* results are reported.

Chemical strategies to increase CNS penetration and stability of opioid peptides

Different approaches, including cationization (e.g. ADAMB), glycosylation and pegylation, have been used to enhance the ability of opioid peptide analogs to penetrate the BBB along with increasing their stability (see ref. [25] for a review). Glycosylation has been suggested to enhance the potency of systemically administered peptides and to promote their penetration across the BBB. Glycosylation of opioid peptides was initially reported over 20 years ago, and since then a variety of glycosylated opioid peptides have been synthesized and examined for opioid activity (see ref. [26]). Recent results with glycosylated opioid peptides, however, suggest that the effects of glycosylation on BBB penetration may be species dependent. The Leu-enkephalin analog MMP-2200, which is a MOR/DOR agonist that contains the disaccharide lactose attached to the side chain of serine at the C-terminus (see Figure 1), produces antinociception activity in mice that is not blocked by the peripherally-restricted opioid antagonist naloxone methiodide, verifying the involvement of central opioid receptors in the antinociceptive activity in this species [27]; MMP-2200 also exhibited less tolerance and less severe withdrawal symptoms compared to morphine. In rhesus monkeys, however, this glycopeptide appears to act as a peripheral MOR/DOR agonist with limited penetration into the CNS [28].

Pegylation has also been explored to enhance the analgesic potency of opioid peptides. In the case of the DOR selective agonist *cyclo*[D-Pen^2,D-Pen^5]enkephalin (DPDPE), which is cleared primarily by biliary excretion, N-terminal pegylation decreases hepatic clearance and enhances analgesia following i.v. administration. This pegylated derivative appears to function as a prodrug, undergoing hydrolysis to the parent peptide [29]. Attachment of 2 kDa poly(ethyleneglycol) (PEG) to the N-termini of the dimeric enkephalin analog biphalin (Figure 1) enhanced antinociceptive activity somewhat following all routes of administration examined (i.c.v., i.v., intramuscular, i.m., and s.c.; see Table 2) [30].

Recently, novel head-to-tail cyclic peptides have been reported that exhibit opioid activity. [YpwFG], a cyclized derivative of endomorphin-1 containing a glycine bridge between Tyr^1 and Phe^4 (Figure 1) synthesized to enhance membrane permeability, exhibits antinociceptive activity in mice following i.p. administration [31]. As expected, cyclization stabilizes the peptide to metabolic degradation by proteases. Studies with the peripherally restricted antagonist naloxone methiodide suggest that at lower doses the peptide produces antinociception predominantly through peripheral MOR, and only at a higher dose (20 mg/kg i.p.) does the peptide activate MOR in the CNS. In our laboratory we synthesized the natural product cyclic tetrapeptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) [32] and found that it exhibits potent antinociceptive activity *in vivo* in the 55° warm water tail withdrawal assay in mice after either central (i.c.v.) (Ross, et al., submitted) or peripheral (i.p.) administration.

Novel opioid peptides with sequences unrelated to the endogenous opioid peptides have also been evaluated in animals (Table 2), and two analogs have undergone clinical trials (see below). An all D tetrapeptide (D-Phe-D-Phe-D-Nle-D-ArgNH$_2$), identified from a combinatorial library, exhibits exceptional selectivity for KOR *in vitro* and appears to be restricted primarily to the periphery after parental administration [33]. Because of the D stereochemistry this peptide should be resistant to metabolism by proteases. Further structural modification resulted in FE 200665 (now known as CR665) and a second analog FE200666 that exhibit exceptional separation of antinociceptive activity from centrally mediated side effects (e.g. sedation) [34]. CR665 and a third generation peptide CR845 have been evaluated in clinical trials (see below).

Opioid peptides examined in humans

Examination of dynorphin fragments and analogs in humans was initiated in the 1980's and 1990's when dynorphin A-(1-13) was evaluated for the treatment of severe pain and suppression of opioid withdrawal symptoms following i.v. and i.t. delivery (see ref. [3] for a review). The Dyn A-(1-8) analog E-2078 given intramuscularly was also evaluated in humans and shown to have analgesic activity comparable to the clinically used small molecule narcotic analgesic pentazocine in surgical patients (see ref. [3]). Recently, a novel dynorphin A-(1-13) derivative CJC-1008, containing a maleimidopropionyl group to promote its covalent attachment to serum albumin and to extend its duration of action, was examined following i.v. infusion in a phase II clinical trial in postherpetic neuralgia patients [35], and found to exhibit analgesic activity for at least 8, but for less than 24, hours.

As noted above, two all D tetrapeptide peripherally selective KOR agonists have undergone clinical trials. CR665 (0.36 mg/kg i.v.) was evaluated in a double blind study on experimental pain involving skin, muscle and viscera, and found to have a selective analgesic effect on visceral pain, consistent with its peripheral KOR selective activity, in contrast to the clinically used narcotic analgesic oxycodone which had a generalized effect on all three types of pain [36]. A third generation analog CR845 has completed a Phase IIa proof-of-concept clinical trial after a single i.v. infusion (0.040 mg/kg) to women following laparoscopic hysterectomy (NCT00877799, http://clinicaltrials.gov) with positive results reported in terms of pain relief and decrease in the incidence of undesirable side effects (Cara Therapeutics press release, February 8, 2010, http://www.caratherapeutics.com/press-releases.php).

In addition to the application of opioid peptides for the treatment of severe pain, the endogenous opioid peptide Met^5-enkephalin (referred to as opioid growth factor, OGF) has been examined in Phase I and II clinical trials for the treatment of advanced pancreatic cancer [37], based on its reported interaction with a target (the OGF receptor) that is distinct from the classic opioid receptors and its inhibition of pancreatic cancer in cell culture and in nude mice. This peptide (0.25 mg/kg i.v.) was reported to provide clinical benefit to 53% of the patients who had failed standard chemotherapy, with a 3-fold increase in survival time. Its efficacy is presently being examined against other cancers (Phase II clinical trials in head and neck cancers, and Phase I clinical trial in hepatocellular cancer; http://clinicaltrials.gov, see Table 2).

A novel approach to treat intractable pain that deserves mention is gene therapy with vectors inducing expression of an endogenous opioid peptide. Replication defective herpes simplex virus-based vectors have been used to express human proenkephalin and an engineered endomorphin-2 gene in animal models, and have been shown to be particularly effective in models of inflammatory and cancer pain. The results of the first small clinical trial with the vector NP2 expressing proenkephalin have just been reported [38], and provided promising results in terms of safety and pain relief.

Conclusions

Peptides have several advantages as potential drugs, including high activity, high specificity, low toxicity, and minimization of drug-drug interactions [39]. However, challenges remain in developing opioid peptides as potential therapeutic agents, particularly in terms of their activity after systemic administration and their ability to cross the BBB (see Box 1). Stabilizing these peptides to metabolism sufficiently to permit systemic administration can be accomplished through a variety of structural modifications (see Figure 1) while still retaining the desired pharmacological activity. The metabolic stability of the peptides can be fine-tuned by the choice and number of modifications introduced. Often multiple

modifications are necessary to produce significant activity following systemic administration, as was the case with many of the peptides discussed here. However, other factors in addition to metabolism, e.g. biliary excretion, can also limit the systemic bioavailability of opioid peptide analogs.

A significant challenge in the development of opioid peptides for clinical use is optimizing their distribution (or lack thereof) into the CNS (see Box 1). As described above, both peptides that can cross the BBB (e.g. Dmt-DALDA and zyklophin) as well as peptides that appear to be restricted to the periphery (e.g. CR665 and CR845) have been identified. The mechanism of action and therapeutic application determines whether it will be necessary for a drug to cross the BBB, or whether activity in the periphery is sufficient and indeed advantageous. For the treatment of pain, agonists that are restricted to the periphery can have distinct advantages because they should not exhibit centrally mediated undesirable side effects (e.g. addiction) that severely limit the clinical use of existing therapeutics. In contrast, for treatment of mood disorders and substance abuse, demonstration that the opioid peptide analogs can cross the BBB is important for the development of these compounds as potential therapeutic agents.

Multiple mechanisms have been implicated in the BBB penetration of opioid peptide analogs [25]. Enhancing peptide lipophilicity can enhance BBB penetration by passive diffusion [25], and the CNS penetration of several MOR selective peptides with different structures was recently correlated with their lipophilicity [40]. However, other mechanisms can be involved in transporting peptides across the BBB (see ref. [25]) or in limiting their CNS penetration (i.e. efflux proteins such as P-glycoprotein [29]). Modifications such as glycosylation or an N-terminal guanidyl group that enhance the hydrophilicity of peptides have also been explored to increase BBB penetration. Unfortunately information on the CNS penetration of opioid peptides is fragmentary and the evaluations performed used a variety of different methods (see ref. [40]), making comparisons difficult and rational design of CNS active peptides challenging. Moreover, apparent species differences in CNS penetration of some peptides (see above, also Box 1) further complicates development of CNS active peptides.

Delivery of therapeutic peptides remains a challenge (see Box 1), but recent advances, e.g. in structural modification strategies, pulmonary delivery [23] and demonstrated activity following oral administration, hold promise that opioid peptides can be systemically administered by a variety of methods including those that don't involve injection to yield therapeutic benefit.

Acknowledgments

Abbreviations

Abbreviations for amino acids follow IUPAC-IUB Joint Commission of Biochemical Nomenclature (*Eur. J. Biochem.* (1984) 138, 9 37). Other abbreviations are

BBB	blood-brain barrier
Cha	cyclohexylalanine
CNS	central nervous system

Dap	2,3-diaminoproprionic acid
Dmt	2′,6′-dimethyltyrosine
DOR	delta opioid receptor
DPDPE	*cyclo*[D-Pen^2,Pen^5]enkephalin
i.c.v	intracerebroventricular
i.m	intramuscular
i.t	intrathecal
i.v	intravenous
KOR	kappa opioid receptor
MOR	mu opioid receptor
Pen	penicillamine
p.o	per oral
s.c	subcutaneous
SAR	structure-activity relationships
Tic	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid

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Dermorphin analogs:

Tyr-D-Ala-Phe-Gly-Tyr-Pro-SerNH$_2$

Dermorphin

Tyr-D-Arg-Phe-MeβAla-OH
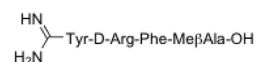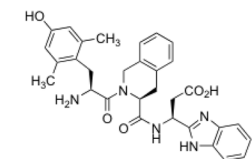
ADAMB

Dmt-D-Arg-Phe-Lys-NH$_2$

Dmt-DALDA

Dmt-D-Arg-Phe-Lys−NH−CH$_2$
Tyr-TicΨ[CH$_2$-NH]Cha-Phe−NH−CH$_2$

Dmt-DALDA-CH$_2$-NH(TICPΨ')

Endomorphin analogs:

Tyr-Pro-Trp-PheNH$_2$

Endomorphin-1

R-Tyr-D-Ala-Trp-Phe(X)NH$_2$

R = guanidino or H

X = p-Cl or H

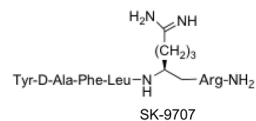Tyr-D-Pro-Gly-Trp-Phe(p-Cl)-NH$_2$

Dmt-Tic derivatives:

UFP-512
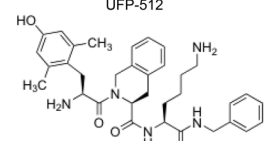
Dmt-Tic-Lys-NH-CH$_2$-Ph
MZ-2

Opioid/neurokinin ligand:

Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Trp−O

TY005
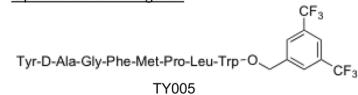
Dynorphin analogs and related peptides:

RTyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-X

	R	X
Dyn A-(1-13)	H	OH
[NMeTyr^1]Dyn A-(1-13)	CH$_3$	OH
" " amide	CH$_3$	NH$_2$

NMeTyr-Gly-Gly-Phe-Leu-Arg-NMeArg-D-Leu-NHEt
E-2078

Tyr-D-Ala-Phe-Leu−N Arg-NH$_2$

SK-9707

Tyr-R-Gly-Phe(p-X)-Met-Lys-Lys-Lys-Phe-Met-Arg-Phe-NH$_2$

	R	X
YFa	Gly	H
[D-Ala^2]YFa	D-Ala	H
[D-Ala^2,Phe(p-Cl^4)]YFa	D-Ala	Cl

[N-Benzyl-Tyr^1,$cyclo$[D-Asp^5,Dap^8]dynorphin A-(1-11)NH$_2$
Zyklophin
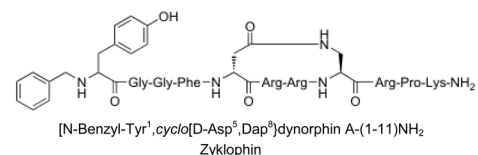
Glycosylated and pegylated peptides:

Tyr-Gly-Gly-Phe-Leu

Leu-enkephalin

Tyr-D-Thr-Gly-Phe-Leu-Ser-NH$_2$
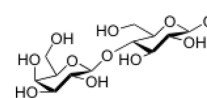
[D-Thr^2]enkephalin-Ser(β-D-lactose)^6 amide
MMP-2200

PEG (2kDa)−(CH$_2$)$_2$−Tyr-D-Pen-Gly-Phe-D-Pen
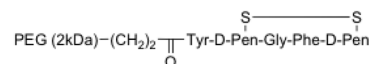
PEG-DPDPE

PEG (2kDa)-Tyr-D-Ala-Gly-Phe−NH
PEG (2kDa)-Tyr-D-Ala-Gly-Phe−NH

Di-PEG-biphalin

Novel opioid peptides:

D-Phe-D-Phe-D-Nle-D-Arg-NHR

c[YpwFG]

	R
FE200041	H
FE2006665 (CR665)	$-CH_2-$ (pyridinyl)

D-Phe-D-Phe-D-Leu-Orn—N(morpholino)

FE200666
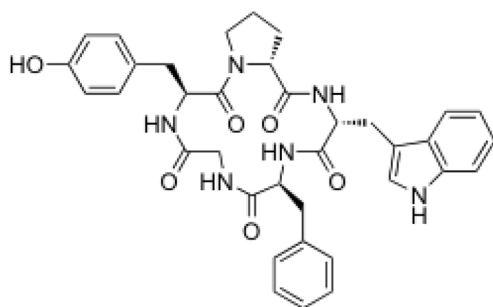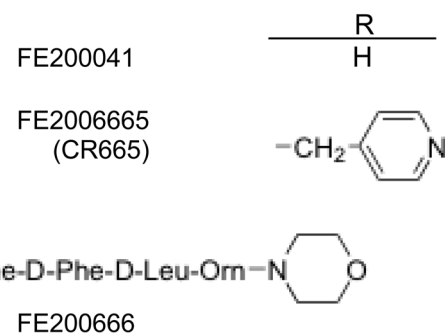
Figure 1.
Structures of opioid peptide analogs exhibiting systemic activity compared to the
endogenous opioid peptides. Structures of CJC-1008 and CR845 were not reported.
Abbreviations used are: Cha, cyclohexylalanine; Dap, 2,3-diaminoproprionic acid; Dmt, 2′,
6′-dimethyltyrosine; DPDPE, *cyclo*[D-Pen^2,D-Pen^5]enkephalin; Pen, penicillamine; Tic,
1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; PEG, poly(ethylene glycol). Ψ[CH$_2$NH]
denotes a reduced amide bond.

Table 1

Potential therapeutic applications currently being explored for opioid peptides targeting different opioid receptors.[a]

Opioid receptor activity	Therapeutic application	Example and reference
MOR agonist[b]	Analgesia	Dmt-DALDA plus several others – see text
DOR agonist	Analgesia; anti-depressant and anxiolytic activity	UFP-512 [13,15]
DOR antagonist Also in combination with MOR agonist activity	Reduction in MOR agonist-induced tolerance	MZ-2 [16] Dmt-DALDA-CH$_2$NH(TICPΨ) [4]
KOR agonist	Peripherally restricted analgesics, especially for visceral and inflammatory pain	CR665 [34, 36] and CR845
KOR antagonist	Treatment for drug abuse (stress-induced reinstatement)	Zyklophin [24]

[a]See ref. [6] for a review of DOR and KOR as drug targets

[b]Also in combination with activity at other receptors, e.g. DOR or KOR agonist activity.

Table 2

Summary of recent studies on systemic activity of opioid peptide analogs in animals and humans. Studies in animals were performed in mice unless otherwise indicated.

Peptide	Opioid receptor activity	Systemic activity	Reference
Systemic activity in animals:			
Dermorphin and endormorphin analogs:			
ADAMB	MOR agonist	$ED_{50} = 0.089$ vs. 3.3 mg/kg s.c., and 5.8 vs. 22 mg/kg p.o for ADAMB vs. morphine in tail pressure test	[8]
Dmt-DALDA	MOR agonist Other effects: inhibits norepinephrine uptake, releases opioid peptides	$ED_{50} = 0.16$ vs. 5.7 μmol/kg s.c. for morphine in radiant heat tail flick assay	See ref. [4]
Dmti-DALDA-CH$_2$NH-(TICPΨ)	MOR agonist/DOR antagonist	$ED_{50} = 5.7$ μmol/kg for both peptide and morphine in tail flick assay	[4]
[D-Ala^2]- and N-terminal guanidino endomorphin-1 derivatives (Figure 1)	MOR agonists	30 mg/kg s.c. produced 40-50% MPE[a] in 50°C warm water tail withdrawal assay	[10]
Dmt-Tic derivatives:			
UFP-512	DOR agonist	Sig. decrease in immobility in forced swim test (antidepressant-like activity) at 1 mg/kg i.p. (mice), 0.3 and 1 mg/kg i.p. (rats) Sig. anxiolytic activity at 1 mg/kg i.p. in light-dark aversion assay and elevated plus maze assays Sig. increase at 10 μg/kg i.p., but sig. decrease at 1 mg/kg i.p. in locomotion in 6-hydroxydopamine lesioned hemiparkinsonian rats	[13] [14] [15]
MZ-2	MOR/DOR antagonist	AD_{50} for antagonism of i.c.v. morphine = 1.6 mg/kg p.o. vs. 0.4 mg/kg for naloxone in hot plate test 0.1 mg/kg s.c. prevented development of morphine tolerance	[16]
		10 mg/kg/day p.o. reduced weight gain and improved other parameters in female *ob/ob* mice	[17]
TY005	MOR/DOR agonist/neurokinin-1 antagonist	3.0 mg/kg i.v. sig. decreased nerve-injury induced tactile and thermal hypersensitivities (rats)	[18]
SK-9709	MOR/KOR agonist	$ED_{50} = 1.4$ vs. 1.1 μmol/kg s.c. for morphine in the acetic acid-induced writhing assay $ED_{50} = 39$ vs. 20 μmol/kg s.c. for morphine in the hot plate test	[19]
YFa	KOR agonist	39 μmol/kg i.p. produced 60% MPE in radiant heat tail flick assay (rats); tolerance did not develop to 26 μmol/kg i.p. dose (rats)	[20]

Peptide	Opioid receptor activity	Systemic activity	Reference
[D-Ala²]YFa [D-Ala²,Phe(p-Cl)⁴]YFa	Agonists at MOR, DOR and KOR	Potency i.p.: [D-Ala²,Phe(p-Cl)⁴]YFa > [D-Ala²]YFa, [Phe(p-Cl)⁴] > YFa ED₅₀ = 14 vs. 36 μmol/kg i.p. for [D-Ala²,Phe(p-Cl)⁴]YFa vs. YFa in radiant heat tail flick assay (rats)	[21, 22]
NMeTyr¹-Dyn A-(1-13) acid and amide	NR[b]	5 μmol/kg i.v. or pulmonary administration, but not s.c., enhanced morphine antinociception in morphine tolerant rats in radiant heat tail flick assay	[23]
Zyklophin	KOR selective antagonist	3 mg/kg s.c. antagonized KOR agonist administered i.c.v. and prevented stress-induced reinstatement of cocaine-seeking behavior in the conditioned place preference assay	[24]
Glycosylated peptide: MMP-2200	MOR/DOR agonist	ED₅₀ = 8.9 vs. 24 μmol/kg s.c. for morphine in 55°C warm water tail withdrawal assay Doses up to 56 mg/kg i.m. or i.v. were ineffective in 50°C tail withdrawal assay (rhesus monkeys); 3.2 mg/kg i.m.. sig. decreased allodynia in capsaicin-induced allodynia assay (rhesus monkeys)	[27] [28]
Pegylated peptides:			
PEG-DPDPE	Prodrug for DOR agonist DPDPE	25 μmol/kg i.v. PEG-DPDPE exhibited sig. higher antinociception than DPDPE in radiant heat tail flick assay	[29]
Di-PEG-biphalin	MOR/DOR agonist	ED₅₀ for di-PEG-biphalin vs. biphalin = 0.24 vs. 0.52 μmol/kg i.v., 4.0 vs. 6.5 μmol/kg s.c. and 5.4 vs. 9.3 μmol/kg i.m. in radiant heat tail flick assay (rats)	[30]
Novel opioid peptides:			
c[YpwFG]	MOR agonist	ED₅₀ = 1.2 mg/kg i.p. and 2.1 mg/kg s.c. in acetic acid-induced writhing assay. Sig antinociception in 52°C warm water tail withdrawal assay only at 20 mg/kg i.p.	[31]
FE200041	Peripheral KOR agonist	ED₅₀ = 0.06 mg/kg i.v. in acetic acid-induced writhing assay (mice); ED₅₀ = 0.39 mg/kg i.v. in formalin-induced flinch assay (rats)	[33]
FE200665 (CR665) and FE200666	Peripheral KOR agonist	ED₅₀ for FE200665 and FE200666 = 0.007 and 0.013 mg/kg i.v., respectively, in mice, and 0.01 and 0.03 mg/kg s.c., respectively, in rats in acetic acid-induced writhing assay; ED₅₀ > 10 and 3.2 mg/kg i.v., respectively, in the 52°C warm water tail withdrawal assay (mice)	[34]
Peptides examined recently in humans[b,c]			

Peptide	Opioid receptor activity	Systemic activity	Reference
CR665	Peripheral KOR agonist	CR665 (0.36 mg/kg i.v.) selectively increased pain rating threshold in visceral pain model (esophageal distension)	[36]
CR845	Peripheral KOR agonist	Completed phase 2a trial, 0.04 mg/kg i.v. in laproscopic hysterectomy patients	NCT00877799[d,e]
CJC-1008	Peripheral agonist	Phase II trial, 3 mg/kg i.v. in postherpetic neuralgia patients; analgesic activity observed for 8, but not 24 hours	[35]
[Met^5]enkephalin (opioid growth factor, OFG)		Phase II trial, 0.25 mg/kg i.v. infusion weekly provided clinical benefit to 53% of advanced pancreatic patients	[37]
		Phase II clinical trials in head and neck cancer (0.25 mg/kg i.v. infusion weekly), phase I clinical trial in hepatocellular cancer	NCT00905099, NCT00982696 NCT00705576[d]

[a]MPE = maximum possible effect; NR = not reported

[b]Early studies examined Dyn A-(1-13) in humans for the treatment of pain and in opiate withdrawal, and E2078 for the treatment of pain (see text and [3]).

[c]A novel approach to using opioid peptides is gene therapy [38] (see text).

[d]See http://clinicaltrials.gov

[e]Cara Therapeutics press release, February 8, 2010, http://www.caratherapeutics.com/press-releases.php